# THE PREPARATION OF REGIOSPECIFIC TRITIATED AND DEUTERATED DIBENZACRIDINES BY CATALYTIC EXCHANGE AND [14-$^{14}$C]DIBENZ-[a,j]ACRIDINE

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## SUMMARY

A practical synthesis of [14-$^{3}$H]dibenz[a,j]acridine and [7-$^{3}$H]dibenz[a,h]acridine is reported from the parent hydrocarbon by catalytic exchange with tritium gas in the presence of palladium on calcium carbonate. Analogous regiospecific labelling with deuterium was also affected. In addition [14-$^{14}$C]dibenz[a,j]acridine was prepared from [$^{14}$C]paraformaldehyde.

KEYWORDS: Dibenzacridines, Tritium, Deuterium, Catalytic Exchange, Carbon-14 Labelling.

## INTRODUCTION

Polycyclic azaaromatic hydrocarbons have been identified as environmental contaminants[1] and as constituents of tobacco smoke condensate,[2] and some have high carcinogenic potency.[1] Studies of the mode of action of such substances involve chemical synthesis of potential metabolites, metabolism studies, and DNA binding studies. Such studies have appeared for benz[a]acridine,[3] benz[c]acridine[3] and 7-methylbenz[c]acridine,[4,5] but for pentacyclic systems related to acridine only synthetic studies have appeared.[6,7] In order to facilitate metabolic and DNA binding studies of dibenz[a,j]acridine (1), dibenz[a,h]acridine (2), and dibenz[c,h]acridine (3), radioactively

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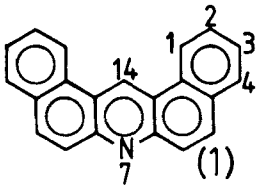
labelled samples were sought with high specific radioactivities. This has been achieved for dibenzacridines 1 and 2.



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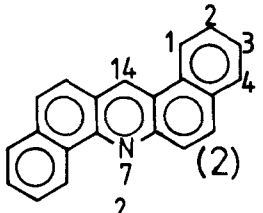

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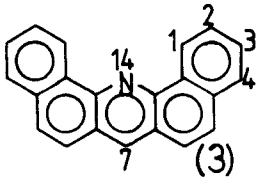

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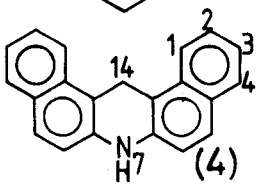

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## EXPERIMENTAL

Synthesis. The dibenzacridines, 1, 2, and 3, were prepared from the appropriate naphthol and naphthylamine by fusion with paraformaldehyde.[8,9]

Labelling - Tritium Labelled Dibenzacridines. Palladium (5%) on calcium carbonate (30mg) was added to a solution of the dibenzacridine (3mg) in dry dioxane (3ml), air was removed on a vacuum system, and the suspension was stirred under tritium gas (5Ci) at a pressure of about 0.3atm at 60° for 1-2h. The catalyst was removed by filtration and the dioxane was removed either by evaporation in a stream of dry nitrogen, or by vacuum-line transfer. The residue was dissolved in methanol (3ml), and the methanol was removed by a stream of nitrogen. This addition and removal of methanol was repeated twice. After the residue was dissolved in methylene dichloride the desired dibenzacridine was isolated by silica gel short column vacuum chromatography, by elution with methylene dichloride/ethyl acetate (10:1). The crude products from the tritiation of dibenz[a,j]acridine and dibenz[c,h]acridine were reoxidised by refluxing in glacial acetic acid (3ml) under air for 6h before isolation by chromatography. The acetic acid was removed by evaporation under reduced pressure and extraction from methylene dichloride solution with 10% aqueous sodium hydroxide.

[14-$^{14}$C]Dibenz[a,j]acridine. This was prepared by the method of Blout and Corley[8] from [$^{14}$C]paraformaldehyde (0.87mg, 579μCi/mg), 2-naphthol (5.3mg) and 2-naphthylamine (4.5mg) by refluxing in xylene (0.3ml) at 140° for 3h. After isolation as described above, the crude product was purified by preparative HPLC on a 5μm Spherisorb column (50cm x 9mm)

using a linear gradient of methanol in water from 80% methanol to 95.8% methanol over 27.5 min, and a flow rate of 5ml/min.

Tritium Nuclear Magnetic Resonance Spectrometry. Spectra were obtained in deuterochloroform solution on a Bruker CXP-300 spectrometer operating at 320 MHz with broad band proton decoupling. Tritium chemical shifts were measured by ghost-referencing from internal non-tritiated TMS.[10]

Determination of Specific Activity and Radiochemical Purity. The incorporation of tritium at only one position was apparent from the appearance of a peak at m/z 282 ($MH^+$) in addition to the expected peak at m/z 280 in the methane ($CH_5^+$) chemical ionization mass spectrum (CIMS) measured on a Finnigan MS3200 E system. The specific activity was determined from the ratio of 282 to 280 peaks.

The radiochemical purity was determined by reverse isotope dilution analysis (RIDA) and by high performance liquid chromatography (HPLC). For HPLC a 10μm Brownlee RP-8 column (25cm x 4.6mm i.d.) and a linear methanol water gradient from 60-80% methanol over 40 min followed by linear 80-100% methanol over 10 min was used. Unlabelled marker compound was added and a flow rate of 1.2ml/min was employed. The retention times of dibenz[a,j]acridine, dibenz[a,h]acridine and dibenz[c,h]acridine were 29.7, 32.8 and 37.4 min, respectively. Fractions (1 min) were collected and radioactivity was determined by liquid scintillation counting.

## RESULTS AND DISCUSSION

The reaction of dibenz[a,j]acridine, 1, with tritium gas gave a product which, before refluxing with acetic acid, showed signals in the CIMS from m/z 280 - 286. However the ion with m/z at 286 in the CIMS was absent in the product isolated after aerial oxidation in acetic acid which possessed its major ion at m/z 282. The appearance of a singlet at 10.42ppm in the tritium NMR of the product, 1, (Table 1) provided evidence that the label was present only at position 14, and the absence of any other signal attests to the regiospecificity of the reaction. This suggested that the isotope incorporation occurred through reversible catalytic reduction of the heterocyclic ring to afford the acridan, 4 (shown without isotope). Catalytic reduction of benz[a]anthracene and 7,12-dimethylbenz[a]anthracene with palladium on carbon has been shown to afford small amount of products reduced only

Table 1: NMR Spectra of Tritiated Dibenzacridines

| Compound | Chemical Shift (ppm) | |
|---|---|---|
| | $^{1}$H-14 (or $^{1}$H-7*) | $^{3}$H-14 (or $^{3}$H-7*) |
| 1 | 10.24 | 10.42 |
| 2 | 9.39 | 9.46 |
| 3 | 8.75 | 8.69 |

* Alternative numbering for compounds 2 and 3.

in the central ring of the anthracene moiety[14] and reduction of 1 has been shown to afford the acridan 4.[8] The incorporation of tritium in 1 was high at about 75% (Table 2).

The reaction for dibenz[a,h]acridine, 2 did not require aerial oxidation and again showed high incorporation of isotope (Table 2). For both 1 and 2 no major radiochemical impurity peaks appeared in the HPLC, and the radiochemical purities measured by both RIDA and HPLC were about 90% (Table 2). For 2 the isotope was again located para

Table 2: $^{3}$H- and $^{2}$H-Labelling of Dibenzacridines

| Compound | Tritium | | | Deuterium |
|---|---|---|---|---|
| | Sp. Act. (Ci/mmol) | Radiochemical Purity (%) RIDA | HPLC | (%) incorporated. |
| 1 | 22.1 | 87.7 | 87.8 | 83.2 |
| 2 | 7.7 | 94.0 | 86.7 | 33.6 |
| 3 | 0.87* | + | 45.6 | 17.5 |

*Determined from HPLC and GCMS

+Not determined

to the nitrogen in the heterocyclic ring as shown by the chemical shifts (Table 1).[11,12] When the reaction for 3 was conducted using only 2Ci of tritium gas and pressures of less than 0.05atm no isotope incorporation occurred. However, at the higher pressure with 5Ci of tritium, labelling occured although at much lower specific activity than with dibenzacridines 1 and 2. A major radiochemical contaminant was dibenz[a,h]acridine which was present at about 9% (by $^{1}$H-NMR) of the [c,h]-isomer, and which could not be removed by repeated recrystallisation of 3 prior to the labelling experiment. The radiochemical impurities were separable by HPLC, and tritium NMR of the

total product showed only one signal due to H-7 in dibenz[c,h]acridine (Table 1).

Similar experiments conducted with deuterium gas at pressures of about 0.3atm afforded deuterated products from all three dibenzacridines. After aerial oxidation by refluxing in glacial acetic acid, the mass spectra showed that only one deuterium atom was incorporated, and that the rank order of ease of deuteration was the same as that found in the tritium exchange experiments with dibenz[a,j]acridine reacting most easily, and its [c,h]-isomer reacting least readily (Table 2). Although the position of the deuterium atom was not proved to be para to the nitrogen, the proton NMR of these products showed relatively weak signals due to residual $^{1}$H-14 (or $^{1}$H-7) which integrated for 8%, 65% and 72% of one proton for compounds 1, 2 and 3, respectively. These values are consistent with deuterium incorporations determined by MS (Table 2) and the presence of the isotope at position 14 (or position 7).

For metabolic work, the dibenzacridines 1 and 2 were purified by preparative HPLC, dissolved in hexane and extracted with 0.075M sodium hydroxide in 42.5% of dimethyl sulfoxide. This afforded material of radiochemical purity better than 97%.

[14-$^{14}$C]Dibenz[a,j]acridine was also prepared by the xylene reflux method from [$^{14}$C]paraformaldehyde, and after preparative HPLC, was at least 97% radiochemically pure by HPLC, and 101.2% pure by RIDA The specific radioactivity was 15.9mCi/mmol. In metabolic work this material has the advantage that formation of dibenz[a,j]acridone is detectable radiochemically whereas such a metabolic pathway with the [14-$^{3}$H]dibenz[a,j]acridine leads to loss of the tritium. This metabolic pathway is important for acridine itself.[15]

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